View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by KhartoumSpace

بسم الله الرحمن الرحيم

Chromogenic Detection of *Salmonella* from Diarrheic Chickens and Antibiotic Sensitivity of the Isolates

By

Gamer Aldawla Mustafa Mohammed Elabbas

BSc, University of Elneelain (2006)

Supervisor

Dr. Elhassan M. A. Saeed

(BVSc, MVSc, PhD)

A dissertation submitted to the University of Khartoum in partial fulfillment of the requirements for the degree of MSc in Microbiology

Department of Microbiology

Faculty of Veterinary Medicine

University of Khartoum

June, 2010

DEDICATION

To those who light their life

candles to enlighten my way…

To my father and kind mother

To my lovely sisters and brothers

To whom they shared with me

The best days in my life in this university…

To my dearest friends

With great love

and respect

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and appreciation to my supervisor Dr. Elhassan M. A. Saeed, the head of the Department of Microbiology for his guidance, valuable suggestions, encouragement and support throughout the entire course of the study.

Thanks are also extended to all the staff and technicians of Microbiology Department, Faculty of Veterinary Medicine, University of Khartoum for their valuable advice and assistance.

Finally, I would like to express my sincere gratitude to my family and all friends who helped generously to make this work possible and to be extended to those I forget to mention in this chance notion.

List of Contents

List of Tables

List of Figures

Abstract

The present study was conducted to detect *Salmonella* species in fecal samples of chickens suffering from diarrhea in Khartoum State, using enrichment medium and Salmonella Chromogenic Medium, during the period from December 2009 to April 2010. A total of 100 cloacal swabs were taken from five open system poultry farms of layers (three farms) and broilers (two farms), 20 samples from each farm. Three farms were located in Khartoum North (Shambt and Alhalfaya localities) and the other two farms were located in Southern Khartoum (Soba and Jabal Awlia localities). Using the Selenite-f-broth as enrichment medium for *Salmonella* and a Chromogenic agar designed specially for *Salmonella* detection, a total of 15 *Salmonella* isolates (15%) were recovered. All of the 15 isolates were obtained from one layer chicken farm from Shambat area. The isolates were identified to the species level according to their cultural and biochemical properties to *Salmonella* Enteritidis. Antibiotic sensitivity test for the 15 *Salmonella* Enteritidis isolates was carried out. Each isolate was tested against ten antibacterial agents using the standard disk diffusion method on Muller and Hinton's Agar medium. The antibiotics used were: Amikacin, Ceftizoxime, Ciprofloxacin, Gentamicin, Ampicillin/Sulbactam, Piperacillin/Tazobactam, Cefotaxime, Chloramphenicol, Tetracycline and Co-trimoxazole. One antimicrobial susceptibility pattern was displayed by the isolates. All of the isolates were sensitive to the antibiotics, except

Tetracycline and Co-trimoxazole, against which they were found to be resistant.

The high isolation rate (75%) of *Salmonella* from one of Shambat farms was considered an outbreak of salmonellosis, which was controlled, through this study, by rapid detection of the causative agent and determination of the appropriate treatment by performing the antibiotic sensitivity test. However, the disease in the other farms did not exist and this reflects the possible normal situation of the disease in chickens in Khartoum State. Because all isolates showed one pattern of antibiotic susceptibility and biochemical reactions, they were considered belong to one strain of *Salmonella* Enteritidis.

المستخلص

الهدف من هذه الدراسة هو الكشف عن وجود بكتريا السالمونيلا في الدجاج الذي يعاني من اسهالات في ولاية الخرطوم، باستعمال وسط مكثر ووسط أجار السالمونيلا الملون وذلك في الفترة من ديسمبر 2009 الي ابريل 2010. أخذت100 عينة (مسحة برازية) من خمسة مزارع نظام مفتوح للدجاج البياض (ثلاث مزارع) واللاحم (مزرعتين) بواقع 20 عينة لكل مزرعة. كانت ثلاث مزارع من الخرطوم بحري (محليتي شمبات والحلفايا) ومزرعتان من جنوب الخرطوم (محليتي سوبا و جبل أولياء). باستخدام وسط السلنايت-ف- السائل كوسط مكثر للسالمونيلا ووسط أجار السالمونيلا الملون المصمم خصيصاً للكشف عن السالمونيلا تم الحصول علي 15 عزلة من بكتريا السالمونيلا بنسبة عزل بلغت 15% من مجموع العينات كانت جميع العزلات من مزرعة دجاج بياض واحده من محلية شمبات. عرفت العزلات لمستوي النوع، اعتماداً علي الخواص المزرعية و الكيموحيوية، بكتريا السالمونيلا الملهبة للأمعاء.

اجري اختبار الحساسية للمضادات الحيوية لجميع العزلات ضد عشرة من مضادات البكتيرية وذلك باستخدام طريقة الانتشار القرصي القياسية في وسط مولار وهنتون. المضادات الحيوية التي تم استخدامها هي: أميكاسين، سفتيزوكزيم، سبروفلوكساسين، جنتاميسين، أمبسلين/سلباكتام، بيبراسيلين/تازوباكتام، سيفوتاكزيم، كلورامفينيكول، تيترسيكلين و كو- ترامكزاول. أظهرت العزلات نمط واحد من الحساسية للمضادات الحيوية حيث وجدت أنها حساسة لجميع المضادات الحيوية ماعدا التيتراسيكلين والكو-ترمكزاول.

أعتبر معدل العزل العالي (75%) في احدي مزارع شمبات، وباءً لمرض السالمونيلا الذي تمت السيطرة عليه من خلال هذه الدراسة بالعزل السريع للعامل المسبب و تحديد الدواء المناسب بأجراء اختبار الحساسية للعزلات. بينما لم يوجد المرض في المزارع الأخري، وهذا ربما يعكس الوضع الطبيعي للمرض في ولاية الخرطوم. نسبة لأن جميع العزلات أبدت نمط واحد من الاستجابة للمضادات الحيوية والاختبارات الكيموحيوية، أعتبرت تابعة لعترة واحدة من بكتريا السالمونيلا الملهبة للأمعاء.

Abstract

The present study was conducted to detect *Salmonella* species in faecal samples of chickens suffering from diarrhoea in Khartoum State, using enrichment medium and salmonella Chromogenic Medium, during December 2009 to April 2010. A total of 100 cloacal swabs were taken from five open system poultry farms of layers (three farms) and broilers (two farms), 20 samples from each farm. Three farms were in Khartoum North (Shambt and Alhalfaya localities) and the other two were in southern Khartoum (Soba and Jabal Awlia localities). Using the Selenite-f-broth as enrichment medium for *Salmonella* spp and a Chromogenic agar designed specially for *Salmonella* spp detection, a total of 15 *Salmonella* isolates (15%) were recovered. All of the 15 isolates were obtained in one layer chicken farm from Shambat area. The isolates were identified to the species level according to their cultural and biochemical properties to *Salmonella* Enteritidis. Antibiotic sensitivity test for the 15 *Salmonella* Enteritidis isolates was carried out. Each isolate was tested against ten antibacterial agents using the standard disk diffusion method on Muller and Hinton's Agar medium. The antibiotics used were amikacin, ceftizoxime, ciprofloxacin, gentamicin, ampicillin\sulbactam, piperacillin\tazobactam, cefotaxime, chloramphenicol, tetracycline and co-trimoxazole. One antimicrobial susceptibility pattern was displayed by the isolates. All of the isolates were sensitive to the antibiotics, except tetracycline and co-trimoxazole.

The high isolation rate (75%) of *Salmonella* from one of Shambat farms was considered an outbreak of salmonellosis, which was controlled by rapid detection of the causative agent and determination of the appropriate treatment by performing the antibiotic sensitivity test. However, the disease did not exist in the other farms, and this may a reflection of the normal situation of the disease in chickens in Khartoum State. Because all isolates showed one pattern of antibiotic susceptibility and biochemical reactions, they were considered as belonging to one strain of *Salmonella* Enteritidis.

المستخلص

هدفت هذه الدراسة للكشف عن وجود بكتيريا السالمونيلا في الدجاج الذي يعاني من اسهالات في ولاية الخرطوم، باستعمال وسط مكثر و وسط أجار السالمونيلا الملون، وذلك في الفترة من ديسمبر 2009 الي ابريل 2010. أخذت 100 عينة (مسحة برازية) من خمس مزارع نظام مفتوح، للدجاج البياض (ثلاث مزارع) واللاحم (مزرعتين)، بواقع 20 عينة لكل مزرعة، ثلاث مزارع من الخرطوم بحري (محليتي شمبات والحلفايا) ومزرعتان من جنوب الخرطوم (محليتي سوبا و جبل أولياء). باستخدام وسط السلنايت-ف-السائل كوسط مكثر للسالمونيلا و وسط أجار السالمونيلا الملون المصمم خصيصاً للكشف عن السالمونيلا تم الحصول علي 15 عزلة من بكتيريا السالمونيلا بنسبة عزل بلغت 15% من مجموع العينات كانت جميع العزلات من مزرعة دجاج بياض واحده من محلية شمبات. عرفت العزلات لمستوي النوع، اعتماداً علي الخواص المزرعية و الكيموحيوية، علي أنها بكتريا السالمونيلا الملهبة للأمعاء.

اجري اختبار الحساسية للمضادات الحيوية لجميع العزلات ضد عشرة من المضادات البكتيرية وذلك باستخدام طريقة الانتشار القرصي القياسية في وسط مولار و هنتون.استخدمت المضادات الحيوية الأتيه أميكاسين، سفتيزوكزيم، سبروفلوكساسين، جنتاميسين، أمبسلين/سلباكتام، بيبراسيلين/تازوباكتام، سيفوتاكزيم، كلورامفينيكول، تيترسيكلين ، كو-ترايموكسازول. أظهرت العزلات نمطاً واحداً من الحساسية للمضادات الحيوية حيث اتضح أنها حساسة لجميع المضادات الحيوية ماعدا التيتراسيكلين والكو-ترايموكسازول.

أعتبر معدل العزل العالي (75%) في احدي مزارع شمبات، وباءً لمرض السالمونيلا و تمت السيطرة عليه أثناء هذه الدراسة بالعزل السريع للعامل المسبب و تحديد الدواء المناسب بأجراء اختبار الحساسية للعزلات. عدم وجود المرض في المزارع الأخري، ربما يعكس الوضع الطبيعي للمرض في ولاية الخرطوم. نسبة لأن جميع العزلات أبدت نمطاً واحداً من الاستجابة للمضادات الحيوية والاختبارات الكيموحيوية، اعتبرت تابعة لعترة واحدة من بكتيريا السالمونيلا الملهبة للأمعاء.

Introduction

Salmonella is a genus of rod-shaped, Gram-negative, non-spore forming, predominantly motile enterobacteria with a size of 0.7 to 1.5 µm x 2.0 to 5.0 µm. *Salmonella* is closely related to the *Escherichia* genus and are found worldwide in warm and cold-blooded animals, in humans, and in non-living habitats. It causes illnesses in humans and many animals, such as typhoid fever, paratyphoid fever, and the food-borne salmonellosis (Ryan and Ray, 2004).

The members of *Salmonella* genus occupy a position of outstanding importance. They not only cause heavy economic losses in domesticated mammals and birds but are also responsible for very many cases of human illness (Khan, 1969). The early observation of the disease was made by Eberth (1880), who described the typhoid bacillus in tissues of a dead patient and the organism was isolated by Salmon in 1885 and named after him (Merchant and Packer, 1967).

Salmonella Pullorum, *S.* Gallinarum and *S.* Enteritidis can infect the ovaries of hens and be transmitted through eggs (Cooper, 1994). Increase in *S*. Enteritidis isolation rate in the north-eastern region of the United States of America identified grade A shell eggs as the dominant vehicle for outbreaks of this infection (Anonymous, 1990). Zhao *et al*. (2001) reported the occurrence of 1.4 million cases of human salmonellosis in the United States of America.

Poultry producers are faced with intensifying pressures from public health authorities, officials and consumers regarding food safety issues (Quinn, 2002).

Due to the at most importance of *Salmonella* detection in man and animals, an accurate and time-saving procedures such as Chromogenic Medium must be used (Manafi, 2000).

The aim of this study was to isolate *Salmonella* from diarrheic chickens in Khartoum State using Salmonella Chromogenic Medium as rapid tool of detection and determination of the antibiotic susceptibility pattern of the isolates.

CHAPTER ONE

LITERATURE REVIEW

1.1 *Salmonella*:

Salmonella is a genus of Gram-negative bacteria belongs to the family enterobacteriaceae. Members of the genus are rod-shaped, singly arranged or in groups. The genus *Salmonella* was named after Daniel Elmer Salmon, an American veterinary pathologist. While, Theobald Smith, was the actual discoverer of the type bacterium (*Salmonella enterica* var. Choleraesuis) in 1885 (Barons, 1996). Smith and Salmon had been searching for the cause of common hog cholera and proposed this organism as the causal agent. Later research, however, would show that this organism (now known as *Salmonella enterica*) rarely causes enteric symptoms in pigs, and was thus not the agent they was seeking (which was eventually shown to be a virus). However, many bacteria in the genus *Salmonella* were eventually shown to cause other important infectious diseases in man and animals (Janda and Abbott, 2006).

1.1.1 Classification:

The scientific classification of *Salmonella* was described by Hafez (2005) as follows:

Domain: *Bacteria,* Kingdom: *Monera,* Phylum: *Protobacteria,* Class: *Gamma Protobacteria,* Order: *Enterobacteriales,* Family: *Enterobacteriaceae,* Genus: *Salmonella.*

The genus *Salmonella* contains more than 2,400 serotypes (Hafez, 2005). Serotyping is based on the Kaufman and White classification scheme in which somatic (O) and flagellar (H) antigens are identified. Occasionally, capsular (Vi) antigens may be detected in a modification of this scheme. Two species are proposed; *S. enterica* and *S. bongori*. *Salmonella enterica* has been divided into six subspecies (Le Minor and Popoff, 1987). The majority of *Salmonellae* of veterinary importance belong to *S. enterica* subspecies *enterica*. The subspecies are further qualified by the serotype to give a final designation such as *S. enterica* subspecies *enterica* serotype Typhimurium (Quinn *et al*., 2008).

The genus *Salmonella* can roughly be classified into 3 groups (Hafez and Jodas, 2000). Group 1 includes highly host adapted and invasive serovars such as *S.* Gallinarum, *S*. Pullorum in poultry and *S*. Typhi in humans. Group 2 includes non-host adapted and invasive serovars such as *S*. Typhimurium, *S*. Arizonae and *S*. Enteritidis. Group 3 contains non-host adapted and non-invasive serovars, most of these serovars are harmless for animals and humans.

1.1.1.1 Antigenic structure:

The antigenic classification of *salmonella* is based on a number of antigens, namely O, H, K, M and (5) antigens. The somatic (O) antigens are polysaccharides that associate with the body of the cell and are designated with Arabic numerals (Buxton and Fraser, 1977). These antigens are heat and alcohol stable (Kauffman, 1966). Serogroups of *Salmonella* are defined by particular somatic antigens; most *Salmonella* isolated in poultry belong to serogroups B, C, or D.

The (H) antigens are determined by flagellar proteins and are both heat and alcohol labile, divided in two phases, 1 and 2, and designated with small letters and Arabic numerals (Williams and Tucker, 1975).

The (K) antigens are capsular or envelope antigens (Kaufman, 1966). A capsular antigen (namely Vi) is discovered by Felix and Pitt (1943). This antigen is destroyed by boiling for 20 minutes.

The (M) antigens are mucoid antigens which are found in mucoid strains of *S*. Paratyphi B (Kaufman, 1966). It is a polysaccharide-nitrogen free and produces more than 4% glucose on hydrolysis (Birch Hirschfield, 1935). The (5) antigen is mucoid antigen and completely destroyed by heating at 120ºC and normal hydrochloric acid. Both *S*. Pullorum and *S*. Gallinarum possess the (O) antigens 1, 9, and 12 (Wilson and Nordholm, 1995).

1.1.2 Characterization:

Poppe and Gyles (1987) examined 185 isolates of 34 serovars of avian *Salmonella* strains for plasmids, drug resistance, biochemical properties, serum resistance and virulence but no serovar other than *S*. Enteritidis, *S*. Typhimurium, and *S*. Heidelberg showed evidence of serovar-associated plasmids. All *S*. Entritidis isolates carried a single plasmid of 36 mega Daltons (MDa), one strain was virulent. Of 27 isolates of *S*. Typhimurium, 11 possessed 60-MDa plasmid and 17 harbored a 2.3 MDa plasmid. Of 24 isolates of *S*. Heidelberg, 21 carried a 2.2 MDa plasmid. The colonization potential of four serotypes of *Salmonella* administered as composite and individually to 1-day-old chicks were studied.

Poppe (1994) conducted study to characterize *S*. Enteritidis strains that were mainly isolated from the environment in Canada. Bio typing indicated that failure to ferment Rhamnose was some times useful as a marker for epidemiologically related strains. Phage type (PT) was the most effective method for subdividing *S*. Enteritidis, it distinguished 12 PTS. Phage type 13 was occasionally associated with septicemia and mortality in chickens.

1.1.3 Cultural characteristics:

Salmonella is facultative anaerobic, the optimum growth temperature is 37ºC, but some growth is observed in a range from 5ºC to 45ºC. *Salmonella* can grow within a pH range of approximately 4.0 to 9.0, with an optimum pH around 7.0 (Cruckishank, 1972). The organism grows in selective enrichment media such as selenite-f-broth and tetrathionate broth, and on differential plating media such as McConkey's, bismuth sulfite and brilliant green agar. The optimum incubation periods for *Salmonella* enrichment cultures were obtained by inoculating enrichment broth onto plating media after 24 hours incubation at 37ºC, after 48 hours at 37ºC, after a 3-day delayed secondary enrichment (DSE) and after a 5-day DSE procedure. Inoculations of enrichment broth onto plating media after 24 hours incubation followed by 5-day (DSE) enables the detection of 96-98% of *Salmonella* positive samples and were the best combination of conditions (Waltman *et al*., 1993).

The *Salmonella* colonies appear with different shapes and colors on different media. On nutrient agar they appear small, smooth, circular and translucent, while *S*. Gallinarum colonies are blue gray. On McConky agar they are colorless, smooth, round, shiny and up to 2 mm in diameter. *S*.

Gallinarum produces colonies larger than *S*. Pullorum and has a characteristic odor. On selenite-f-broth the growth is turbid with heavy flocculent sediment.

On Desoxycholate Citrate Agar (DCA) the colonies are slightly opaque, dome-shaped with central black spot. *S*. Pullorum is a slow lactose fermenter producing pink colonies with a precipitate in surrounding media. On Triple Sugar Iron Agar (TSI), *S*. Pullorum and *S*. Gallinarum produce a red slant with yellow butt that show delayed blacking from H_2S production.

1.1.3.1 Differential and selective solid media:

There are several selective plating media for the isolation of *Salmonella* from human feces and other specimens. Their specificity and sensitivity vary considerably, these include:

1.1.3.1.1 Taylor's Xylose Lysine Desoxycholate (XLD):

It is a popular medium for the primary plating of feces from suspected *Salmonella* and *Shigella* infections. It gives colony appearances that distinguish *Salmonella* from *Shigella*, and these pathogens from the many non-lactose fermenting strains of non-pathogenic enterobacteria which form pale colonies similar to theirs on McConkey and DCA. Colonies of *Salmonella* and *Shigella* are red (alkaline to phenol red) because *Shigella* does not form acid from xylose, lactose and sucrose in the medium within 24 hrs and because *Salmonella* neutralizes the acid that forms from the limited amount of xylose by decarboxylating the lysine. Most *Salmonella* and *Edwardsiella* are distinguished from the *Shigella* because they produce

hydrogen sulphide, which reacts with ferric ammonium citrate in the medium to produce black centers in their red colonies (Cheesbrough, 2000).

1.1.3.1.2 Wilson and Blair's Brilliant-Green Bismuth Sulphite Agar (BBSA):

This medium is particularly valuable for the isolation of *S.* Typhi. Cultures should be examined after 24 hrs, then after 48 hrs. Crowded colonies about 1 mm in diameter may appear green or pale-brown. Larger discrete colonies have a black center and clear edge (Barrow and Feltham, 1993).

1.1.3.1.3 Leifson’s Desoxycholate-Citrate Agar (DCA):

The colonies of *Salmonella* on DCA are similar to or slightly smaller in size than those on McConkey agar. They are pale, nearly colorless, smooth, shiny and translucent. Sometimes they have a black center and sometimes a zone of cleared medium surrounds them, but these characters may required 48 hrs of incubation for their development. *Salmonella* is easily distinguished from the opaque pink colonies of lactose-fermenting coliform bacilli, which are largely inhibited on this selective differential medium (Cheesbrough, 2000).

1.1.3.1.4 Brilliant Green McConkey Agar:

The addition to McConkey agar of brilliant green 0.004 g\liter, which is inhibitory to *E. coli*, *Proteus* species and the other commensal enterobacteria likely to out number the *Salmonella* in feces, makes this an excellent selective as well as differential medium for *Salmonella* except *S.* Typhi which is not grow well on it. *Salmonella* appears as low convex, pale-

green translucent colonies, 1-3 mm in diameter. Lactose fermenting bacteria, including rare strains of *Salmonella* serotypes, produce blue-purple colonies (Murray, 2005).

1.1.3.1.5 McConkey Bile-Salt Lactose Agar:

After 18 to 24 hrs the colonies are pale yellow or nearly colorless, 1-3 mm in diameter, and easily distinguished from the pink-red colonies of lactose fermenting commensal coliform bacilli, e.g. *Escherichia coli* which also grows well on this unselective differential (indicator) medium (Koneman *et al.,*1997).

1.1.3.2 Salmonella chromogenic media:

Chromogenic media are a some what recent development in the life sciences, having first been used in the late 1980s when the possibilities of selective detection of microorganisms through chromogenic reactions came to be recognized (Poupart *et al.,* 1991). Since 1990, a wide range of chromogenic culture media has been made commercially available by many companies (Oxoid, Merk, Conda…ect.) providing useful tools for diagnostic clinical microbiology, by the inclusion of chromogenic enzyme substrates such as x-glucuronide, x-*β*-D-glucuronide, Salmon-Gal, x-*β*-glucuronide, x-Gal, Magenta-caprylate, x-*β*-glucoside and x-glucoside, targeting microbial enzymes such as gluconidase, *β*-D-glactosidase, *β*-glucosidase, *α*-D-glucosidase and *α*-glucosidase, the enzymes cleave specific substrates to give visible distinguish colors (Miles *et al*., 1992). These media are able to target pathogens with high specificity, for examples; *Staphylococcus aureus*, *Streptococcus agalactiae*, *Salmonella* species and *Candida* species. The inclusion of multiple chromogenic substrates into culture media facilitates

the differentiation of polymicrobial cultures, thus allowing for the development of improved media for diagnosis of e.g., urinary tract infections and media for the enhancing discrimination of yeasts. This procedure is often used as a simple test for the presence or absence of a particular microorganism, but along with other materials or by plating the media, they can also be used for the quantitative analysis of microbes (Aamlid *et al.,* 1989).

Several studies show that newly chromogenic media such as Oxoid Salmonella Precis, Merck Salmonella Chromogenic Media and Rambach's Agar, which have been developed for the detection of *Salmonella* species, have a higher specificity than conventional media. Some are also reported to have a higher sensitivity (Manafi, 2000). Salmonella chromogenic agar is a selective Chromogenic Medium, used for the detection and presumptive identification of *Salmonella* species from clinical samples, food and water. The conventional media used to differentiate species of *Salmonella* from the rest of *Enterobacteriaceae* family, based on their capacity to produce hydrogen sulfide associated with their inability to ferment lactose, are not really adequate as there are about 2500 species of *Salmonella* which do not have these characteristics. Casein peptone and beef extract are the nutrient sources of nitrogen, vitamins, amino acids and minerals. The Chromogenic mixture aids in inhibiting Gram-positive organisms, *Proteus* and *Coliforms*. To identify *Salmonella* species, Salmonella chromogenic agar is based on the combination of two chromogenic substrates that ease quick identification. These two chromogenes are X-gal and Magenta-caprylate. X-gal is a substrate incorporated to visualize the enzyme *ß*-D-galactosidase-producing organisms as blue colonies. Magenta colonies are a result of the

hydrolysis of Magenta-caprylate by the *Salmonella* species. Thus, non-*Salmonella* organisms appear blue or are not stained by any of the chromogenes of the medium (Odonoughe, 1993).

Five selective media for the isolation of *Salmonella* species have early been described (Ruiz *et al*., 1996): Rambach agar, SM-ID medium, xylose-lysine-Tergitol 4 agar, novobiocinbrilliant green-glycerol-lactose agar and modified semisolid Rappaport Vassiliadis medium. In comparison with established media, these formulations promise facilitated recovery of *Salmonella* because of either higher sensitivity (percentage of *Salmonella*-positive stools that yield *Salmonella* species, on a particular medium) or higher specificity (percentage of plates with colonies resembling *Salmonella* species, from which *salmonella* is isolated).

1.1.4 Biochemical characteristics:

The genus *Salmonella* produces usually gas from glucose except *S*. Typhi which ferments glucose and mannitol without gas production (Cruickshank, 1972). Hydrogen sulphide is usually produced on triple sugar iron agar but some strains of *S*. Choleraesuis and most strains of *S*. Paratyphi A do not, and *S*. Arizonae utilizes manitol.

Nitrate reduced to nitrite and citrate is usually utilized by *Salmonella* as a carbon source (Minor, 1984). The members are urease, indole and oxidase negative but catalase positive (Cruickishank, 1972). Sucrose, salicin and lactose are not generally fermented by *Salmonella*. However, many strains of *S*. Arizonae have activity to *β*-glactosidase enzyme (Holt *et al*., 1994).

1.1.5 Serological tests:

These are satisfactory for establishing the presence and estimating the prevalence of the infection within a flock. The tests that are readily applied include tube agglutination test, rapid serum agglutination test, stained antigen whole blood test and micro-agglutinationtest (Gast, 1997). Other serological tests include micro-antiglobulin (Coomb's), immunodiffusion, haemagglutination and enzyme linked immunosorbent assay (ELISA).

The rapid serum agglutination test can be used under field conditions and the reactors can be identified immediately. Chickens can be tested at any age, although some authorities specify a minimum age of 4 months (Wray and Wray, 2000).

1.1.6 Pathogenicity of *Salmonella*:

Three toxins (endotoxin, enterotoxin and cytotoxin) play a role in the pathogenicity of *Salmonella*. The endotoxin produces fever, the enterotoxin causes mucosal damage in cell culture and the cytotoxin inhibits protein synthesis (Koo *et al*., 1984).

1.1.7 Salmonellosis in poultry:

Salmonella Pullorum, *S*. Gallinarum and *S*. Enteritidis can infect the ovaries of hens and be transmitted through eggs. The presence of *S*. Enteritidis in undercooked egg dishes may result in human food poisoning (Cooper, 1994).

Jardy and Michard (1992) tested samples of raw poultry feed components for *Salmonella.* The most commonly isolated serovars were *S*.

Senftenbeg, *S*. Rissen, *S*. Tennessee, *S*. Iandott, *S*. Mbandaka, *S*. Agona and *S*. Havana.

In Iraq, Al Obaidi *et al*. (1992) sampled dead-in-shell embryos from 4 local hatcheries to investigate the causative pathogenic microorganisms. They recovered 35 isolates representing 8 bacterial genera which included *Klebsiella, Proteus, Escherichia coli, Staphylococcus, Salmonella, Shigella, Pseudomonas and Streptococcus.*

Orhan and Guler (1993) isolated *Salmonella* from internal organs, cloacal swabs, feed samples and eggs. These strains were identified as *S*. Gallinarum (25), *S*. Enteritidis (13). While, Pan *et al*. (1993) reported the isolation of 63 strains of *S*. Pullorum.

In Canada, Poppe (1994) reported the isolation of *S*. Enteritidis from samples taken from liver, heart, gizzard, small intestine and cloaca.

In Sudan, Ezdihar (1996) examined 610 samples from infected chickens and reported the isolation of 14 bacterial genera which included *Klebsiella, Citrobacter, E.coli, Salmonella, Enterobacter*, *Proteus, Yersinia, Edwardseille, Serratia, Morganella, Hafnia, Acitobacter and Shigella*.

1.1.8 Incidence of *Salmonella* in man:

In France, *Salmonella* is one of the major sources of toxin infection in humans (Bouvet *et al*., 2002). The incidence of human salmonellosis has increased greatly over the past 20 years and this can mostly be attributed to epidemics of *S.* Enteritidis in poultry in numerous countries (Barrow *et al*., 2003). The association between egg consumption and *S*. Enteritidis

outbreaks is a serious international economic and public health problem (CDC, 2000).

In 1985, 46 (92 %) of 50 reported *Salmonella* food-borne outbreaks in Spain were caused by *S*. Enteritidis, and eggs and egg-containing foods were the most commonly implicated vehicles (Munnich, 1973). Epidemiologic investigations of *S*. Enteritidis outbreaks in France have implicated a wide variety of egg-containing vehicles ranging from an asparagus egg sauce to chocolate mousse (Nurmi and Rantala, 1973). In Norway an outbreak of *S*. Enteritidis infections involving 16 children and 4 adults was traced to consumption of a lemon mousse made from raw eggs (Seuna and Nurmi, 1997). In the United Kingdom, poultry as well as eggs are associated with the increase in *S*. Enteritidis infections; the majority of *S*. Enteritidis isolated from humans in England, Scotland, and Wales are of the same phage type 4, as are the *S*. Enteritidis isolated from both poultry and eggs (Anonymous, 1990).

Eggs have long been associated with *Salmonella* infections (William and Tucker, 1975). They can become contaminated after contact with chicken feces if pores or cracks in the shell allow bacteria entry. A second mode of contamination has been demonstrated for several serotypes; an ovarian or oviduct infection can contaminate egg contents before the shell is formed around the yolk and albumin (Madden, 1990). If infected primary poultry breeding flocks can transmit *S*. Enteritidis infections to their progeny via trans ovarian route, and the progeny subsequently lay contaminated eggs, then commercial egg quality control measures that merely focus on elimination of cracked eggs or external sanitation of eggs can not fully protect the consumer. Furthermore, primary prevention by embargoing eggs

from symptomatically infected flocks may not be effective since this organism appears to be host-adapted and the infected chickens usually do not demonstrate any clinical illness (Sharp, 1988).

The transmission of *Salmonella* species is usually associated with consumption of contaminated food (Soumet *et al*., 1999). However, a great number of outbreaks might be associated with contaminated water, which is known to be an important transmission route (Fertado *et al*., 1998).

1.1.9 Control and treatment:

For several years the control of *Salmonella* species in poultry has been a high priority of the U.S. Department of Health and Human Services and the Agricultural Research Service (Bailey, 1988). Much of the effort has focused on exclusion or reduction in the numbers of *salmonella* by introducing competitive micro floras and by hygienic processing of feed (Bailey, 1988).

Control of *Salmonella* can also be achieved by administration of antimicrobial agents in the drinking water (Nurmi and Rantala, 1973) and by combined therapy involving antimicrobial agents and competitive flora (Seuna and Nurmi, 1980). Williams and Tucker (1975) reduced the number of *S.* Typhimurium cells in experimentally infected chickens by using a combination of Trimethoprim and Sulfadiazine. Trimethoprim and Sulfamethoxazole have been recommended for treatment of salmonellosis in humans (Munnich, 1973).

Seuna and Nurmi (1980) showed that combined therapy involving bacterial cultures and certain antimicrobial agents, including

Oxytetracycline, Neomycin, Polymyxin, and Trimethoprim, significantly reduced the infection rate in chickens by *S.* Infantis, while the bacterial cultures alone only had a slight anti-salmonella effect. Antibiotic treatment followed by feeding of bacterial cultures helped prevent reappearance of infections (Seuna and Nurmi, 1980).

The administration of injectable antibiotics as Gentamicin in the hatchery played a vital role in controlling the spread of *S*. Arizonae in turkey pouts (Shivaprasad *et al*., 1998). Antibiotics have been used to control *S*. Enteritidis infection in several experimental and commercial contexts. Treatment of chicks with both Polymyxin B Sulphate and Trimethoprim prevent and cleared experimental infection (Goodnough and Johnson, 1991). Administration of Flavophospholipol or Salinomycin sodium as feed additives reduced fecal shedding (Bolder *et al*., 1990). Provision of competitive exclusion culture to restore a protective normal micro-flora after treatment with Enrofloxacin reduced the isolation of *S*. Entertidis from broiler breeders and their environment (Reynolds *et al*., 1997).

To control this zoonosis, a number of prophylactic means have been developed. Vaccinations have a general effect and may reduce animal contamination and rate of excretion of bacterium through the feces (Zhang-Barber and Turner, 1999). Other methods aim to reduce the introduction of the bacterium into the gut, which is based on the early implementation of an adult-type intestinal flora which competes with *S*. Enteritidis (Rabsch *et al*., 2000). Genetic methods may also be successful in increasing resistance to systemic disease (Barrow and Feltham, 1993) or carrier-state (Beaumonal *et al*., 1999) thus reducing the need for antibiotic treatments and the risk of antibiotic resistance.

1.1.10 Drug susceptibility:

An increase of *Salmonella* strains showing resistance and multiple resistance against different antibiotics have been found from isolates from poultry in recent years. Kheir Eldin *et al.* (1987) examined, *in vitro*, the sensitivity of 89 isolates of *S*. Gallinarum, *S*. Pullorum, *S.* Virchow and *S*. Newport against 11 antibiotics. The results revealed that 70-80% of the isolates were sensitive to Flumaquine and Chloramphenicol, and that 38-57% were moderately sensitive to Nitrofurantoin, Ampicillin and Neomycin and only 15-18% were weakly sensitive to Lincomycin and Streptomycin, but completely resistant to Erythromycin, Penicillin, Tetracycline and Trimethoprim.

Roliniski *et al*. (1994) determined that 52.98% of *S*. Enteritidis and *S*. Typhimurium were resistant to Nitrofurans, Oxytetracycline, Sulphanomides alone and with Trimethoprim. Similar levels of resistance (49.84%) were shown by *S*. Gallinarum isolates to Oxytetracycline and Sulphanoamides alone and with Trimethoprim and only 8% were resistant to Nitrofurans.

Esaki *et al*. (2004) isolated 94 *Salmonella* strains of 10 serotypes from different poultry farms in Chile (broiler and layer hens). Thirty-nine of them were resistant to Flumaquine, Nalidixic acid and Oxolinic acid. All strains were sensitive to Ciprofloxacin. The most frequent serotypes were *S*. Enteritidis and *S*. Heidelberg.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Sampling:

2.1.1 Source of samples:

The samples were taken from open system chicken farms (layers and broilers) in Khartoum State. Cloacal swab samples were obtained from two broiler and three layer farms during the period from December 2009 to April 2010. One of the two broiler farms was located in Shambat area (a farm in Faculty of Animal production, University of Khartoum) and the second was located in Jabal Awlia. While, the three layer farms were located in Alhalfaya, Shambat and Soba areas.

2.1.2 Sample size:

A total of 100 cloacal swabs were collected, from the five farms as 20 samples from each farm.

2.1.3 Transport and storage of samples:

After cloacal swabbing, using sterile swabs, of diarrheic chickens, all samples were placed on ice in a thermos flask immediately after collection and transported to the Laboratory of Bacteriology of the Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum and kept at 4ºC, before being processed as soon as possible.

2.2 Bacteriological investigation

2.2.1 Sterilization procedures:

2.2.1.1 Hot air oven:

Glassware (flasks, test tubes, pipettes and petri-dishes) was sterilized in hot air oven at 160ºC for 1 hour.

2.2.1.2 Autoclaving:

Autoclaving was used to sterilize culture media and solutions at 121ºC for 15 minutes.

2.2.1.3 Disinfection:

Alcohol (70%) was used to disinfect the surfaces of working benches before and after use.

2.2.1.4 U.V. Light:

It was used to sterilize the vacuum, benches and walls of media pouring room.

2.2.2 Culture media:

2.2.2.1 Liquid media:

2.2.2.1.1 Selenite-f-broth (Oxoid Ltd, England):

According to manufacturer, the medium was prepared by dissolving 23 grams of the dehydrated medium (5.0g peptone, 4.0g mannitol, 10g disodium hydrogen phosphate and 4.0g sodium hydrogen selenite) in one liter of distilled water. The pH was adjusted to 7.0. The medium was dispensed into sterile test tubes as 10 ml amounts before being sterilized by steaming for 20 minutes.

2.2.2.1.2 Sugar fermentation media:

The sugar media composed of peptone water and different sugars. The pH of the peptone water (900 ml) was adjusted to 7.1-7.3 before 10 ml of Andrade's indicator was added, then 100 ml of 10% sugars solution (glucose, mannitol, sucrose, salicin, malonate, raffinose, xylose, lactose, sorbitol, Rhamnose, dulcitol and maltose) were added to the mixture, then it was mixed well and distributed in to 2 ml mounts into sterile test tubes containing inverted Durham's tube, then sterilized by autoclaving at 121°C for 30 minutes and stored refrigerated until use.

2.2.2.2 Semi-solid media:

2.2.2.2.1 Motility medium (Oxoid Ltd. England):

This medium was prepared by adding 13 grams of nutrient broth and 7.5 g of Oxoid agar No.1 to one liter of distilled water and dissolved by boiling. The pH was adjusted to 7.2 and then poured into test tubes, containing Craigie tubes, as 5 ml per tube. The tubes were sterilized by autoclaving at 121ºC for 15 minutes, and cooled to use for motility test.

2.2.2.2.2 Triple sugar iron agar medium (TSI) (Oxoid):

Triple sugar iron agar was prepared by adding 65g of dehydrated medium to one liter of distilled water and dissolved by boiling, the pH was adjusted to 7.4 and then poured into McCarteny bottles 5 ml per bottle, then sterilized by autoclaving at 121ºC for 15 minutes. The medium was allowed to set in slope position about one inch butt and stored refrigerated.

2.2.2.3 Solid media:

2.2.2.3.1 Salmonella chromogenic medium (Conda Ltd. Spain):

Thirty-seven point one grams of the dehydrated medium were suspended in one liter of distilled water and then boiled for one minute. The medium was poured into Petri-dishes as 15-20 ml amounts. The plates were kept refrigerated and protected from light.

2.2.2.3.2 Nutrient agar (Oxoid Ltd. England):

Twenty-eight grams of the dehydrated medium were suspended in one liter of distilled water, mixed well and then dissolved by boiling. The pH was adjusted to 7.2. Then was sterilized at 121°C for 15 minutes, before being dispensed into petri-dishes as 15-20 ml portions.

2.2.2.3.3 Muller Hinton's Agar (Conda Ltd. Spain):

Thirty-eight grams of the dehydrated medium were suspended in one liter of distilled water, mixed well and then dissolved by boiling. The pH was adjusted to 7.2. Then it was sterilized at121°C for 15 minutes, before being dispensed into petri-dishes as 15-20 ml portions.

2.2.3 Solutions:

2.2.3.1 Normal saline:

This solution was prepared by dissolving 8.5 grams of sodium chloride in one liter of distilled water (Cowan and Steel, 1985). The solution was distributed into test tubes as 5 ml per tube and sterilized by autoclaving at 121ºC for 15 minutes.

2.2.4 Cultivation of samples:

2.2.4.1 Inoculation of enrichment medium:

Cloacal swabs were inoculated into medical bottles containing 100 ml of selenite-f-broth and then incubated aerobically at 37ºC for 6-8 hours.

2.2.4.2 Inoculation of plates:

A loop of the selenite-f-broth culture was streaked by the four guardant fashions on a plate of Salmonella Chromogenic Medium and incubated aerobically at 37ºC for 18-24 hours.

2.2.5 Isolation and purification:

Discrete colonies of *Salmonella*-like (magenta-colored colonies) from the primary culture were sub-cultured onto nutrient agar plates and incubated aerobically at 37ºC for 18-24 hours to ensure purification.

2.2.6 Identification of the isolates (Cowan and Steel, 1985):

2.2.6.1 Microscopic examination:

2.2.6.1.1 Gram's stain:

Smears were prepared from the pure culture of each isolate by emulsifying a part of a colony in a drop of sterile normal saline on a glass slide, dried and fixed by heating. Then the slides were flooded by crystal violet for one minute and then washed with tap water. Iodine solution was applied for one minute, and then the slides were washed with tap water. The smear was then decolorized with few drops of acetone for seconds and washed immediately with water. Then the smear was flooded with diluted carbol fuchsin for 30 seconds and washed with tap water. Slides were then

blotted dry with filter paper and examined under oil immersion lens. Singly arranged, Gram-negative short rods were considered for *Salmonella*.

2.2.6.1.2 Motility test:

Motility medium was inoculated at the top of one end of the Craigie tube with tested organism and incubated at 37ºC for 24h. Positive test was indicated by presence of growth out side the Craigie tube.

2.2.6.2 Biochemical testing:

2.2.6.2.1 Sugar fermentation tests:

The sugar fermentation medium was inoculated with the test organism and incubated at 37ºC overnight. It was examined daily for seven days. Acid production was indicated by development of pink color in the medium; gas production was indicated by air trapped in the Durham's tube. The sugars used in these tests were lactose, salicin, sucrose, maltose, mannitol, raffinose, inositol, xylose, malonate, dulcitol, glucose, rhamnose and sorbitol.

2.2.6.2.3 Hydrogen sulphide (H_2S) production:

The *Salmonella* isolates were inoculated by stabbing the butt and streaking the slope of triple sugar iron agar in McCarteny bottles and incubated at 37ºC for 1 day. A positive reaction was indicated by development of a black color.

2.2.7 Antibiotic sensitivity test:

Sensitivity of *Salmonella* isolates to a number of antibacterial agents (Table 1) was determined by the standard disk diffusion method (Buxton and Fraser, 1977). Each isolate was tested against ten antibacterial agents that are mainly used against Gram-negative bacteria. A few colonies from each isolate were emulsified in 2 ml nutrient broth in a test tube and shaken thoroughly to obtain a homogenous suspension of the test culture. A Muller Hinton's plate was then flooded with the bacterial suspension, tipped in different directions to cover the whole surface with the suspension. Excess fluid was aspirated and the plates were left for 15 minutes to dry. The antibacterial disks were placed on the agar medium by using sterile forceps. The plates were then incubated at 37ºC and examined after 24 hours for zones of inhibition which were measured in millimeters. The isolates were described as resistant, intermediate or sensitive to antibacterial agents according to Bauer *et al*. (1966) (Table 2).

Table (1): Antibacterial drugs used in antibacterial sensitivity test

Antibacterial agents	Code	Content
Ampicillin/Sulbactam	AS	200/20 mcg
Co-Trimoxazole	BA	25 mcg
Cefotaxime	CF	30 mcg
Piperacillin/Tazobactam	TZP	100/10 mcg
Chloramphenicol	CH	30 mcg
Ciprofloxacin	CP	5 mcg
Ceftizoxime	CI	30 mcg
Tetracycline	TE	30 mcg
Gentamicin	GM	10 mcg
Amikacin	AK	30 mcg

Table (2): Standard zone of inhibition to used antibacterial agents

Antibacterial agents	Zone of inhibition (Diameter in mm)		
	Resistant	Intermediate	Sensitive
Ampicillin/Sulbactam	11 or less	12- 14	15 or more
Co-trimoxazole	10 or less	11- 15	16 or more
Cefotaxime	14 or less	15- 22	23 or more
Piperacillin/Tazobactam	17 or less	18- 20	21 or more
Chloramphenicol	12 or less	13- 17	18 or more
Ciprofloxacin	15 or less	16- 20	21 or more
Ceftizoxime	14 or less	15- 19	20 or more
Tetracycline	14 or less	15- 18	19 or more
Gentamicin	13 or less	14- 15	16 or more
Amikacin	14 or less	15- 16	17 or more

CHAPTER THREE

RESULTS

3.1 Isolation of *Salmonella*:

A total of 100 samples were subjected to bacteriological examinations using selenite-f-broth and Salmonella Chromogenic Medium. Only 15 isolates with characteristics consistent with *Salmonella* were recovered, 85 samples showed no bacterial growth.

3.2 Origin of positive samples:

All of the 15 isolates of *Salmonella* were recovered from the samples obtained from the farm of layer chickens in Shambat locality, with a detection rate of 75%.

3.3 Identification of the isolates to the species level:

According to motility and sugar fermentation tests, the *Salmonella* isolates were all identified as *S*. Enteritidis.

3.3.1 Properties of *Salmonella* isolates:

3.3.1.1 Cultural properties:

3.3.1.1.1 Growth in selenite-f-broth medium:

Growth in selenite-f-broth was detected by brown precipitate in the medium after 6-8 hours of incubation at 37ºC, in case of only 15 samples.

3.3.1.1.2 Growth in semi-solid motility medium:

In motility medium, all *Salmonella* isolates defused from the site of inoculation, a result indicated that the isolates were motile.

3.3.1.1. Growth on solid media:

3.3.1.1.3.1 Growth on Salmonella chromogenic medium:

On Salmonella Chromogenic Medium, *Salmonella* colonies appeared magenta in color, moderately large, circular and with smooth surface (Figure 1).

3.3.1.1.3.2 Growth on nutrient agar medium:

On nutrient agar medium, *Salmonella* colonies appeared moderately large, circular with smooth surface and grayish-white in color.

3.3.2 Microscopic properties:

All *Salmonella* isolates were Gram-negative, singly arranged and short slender rods.

3.3.3 Biochemical tests:

3.3.3.1 Sugar fermentation results:

Salmonella isolates fermented glucose, with gas production, fermented mannitol, rhamnose, dulcitol and maltose, with out gas production and did not ferment sucrose, salicin, malonate, raffinose, xylose, lactose, inositol and sorbitol (Table 3).

3.3.3.2 Hydrogen sulphide (H_2S) production:

On triple sugar iron agar, *Salmonella* isolates produced hydrogen sulphide which was indicated by black color, gas production causes cracks in the agar and acid pH which was indicated by production of pink color in the slant.

3.3.4 Sensitivity of isolated *Salmonella* to antibacterial agents:

Sensitivity test to the 15 *Salmonella* isolates against ten antibacterial agents was carried out. All isolates were found sensitive to Amikacin, Ceftizoxime, Ciprofloxacin, Gentamicin, Ampicillin\Sulbactam, Piperacillin\Tazobactam, Cefotaxime and Chloramphenicol, while all of them were resistant to Tetracycline and Co-trimoxazole.

Table (3): Biochemical testing of *Salmonella* isolates

Biochemical test	***Salmonella* Enteritidis**
Glucose	+
Mannitol	+
Dulcitol	+
Sorbitol	-
Rhamnose	+
Raffinose	-
Malonate	-
Maltose	+
Xylose	-
Sucrose	-
Salicin	-
Lactose	-
Inositol	-
H_2S	+

Figure (1) Growth of *Salmonella* on Salmonella Chromogenic Medium.

CHAPTER FOUR

DISCUSSION

Salmonellosis in poultry is a worldwide economic and public health problem as *Salmonella* is a major cause of human food-poisoning and causes huge losses in poultry farms (Quinn *et al.,* 2008). This study was carried out to isolate *Salmonella* from diarrheic chickens in Khartoum State, using Salmonella Chromogenic Medium as a rapid tool of detection.

Out of five chicken farms in remote areas of the State, *Salmonella* was found in only one farm with a detection rate of 75% (15 samples out of 20). This finding may indicate that *Salmonella* infections in chicken farms of Khartoum State are sporadic in nature and the infection in the positive farm is an outbreak. This high detection rate of the positive farm was not found reported before in this country. However, many outbreaks in the world were reported before in chickens due to this organism (Anonymous, 1990). Many studies were conducted before in Khartoum State and some other parts of the Sudan, e.g., Yagoab and Mohammed (1987), Ezdiher (1996), Hiba (2007) and Hisham, (2010) and their *Salmonella* detection rate varied between 2.9% and 7.4%, which is considered higher compered to the four negative farms and much lesser than the detection rate of the positive farm.

The 15 isolates of *Salmonella* were identified as *Salmonella* Enteritidis. This species was isolated before from chickens in Khartoum North (Hisham, 2010). So, its isolation is not unexpected. This species was also worldwide reported to be isolated from chickens (Anonymous, 1990; Orhan and Guler, 1993; Seuna and Nurmi, 1997 and Barrow *et al*., 2003). Non-*Salmonella* diarrhea causative agents are suggested to be the cause of

diarrhea in the four negative farms. Other bacteria, e.g., *E. coli, Shigella, Klebsiella, Citrobacte rand Enterobacter* and parasites, especially protozoa may be incriminated. The serovar *Salmonella* Enteritidis is highly invasive and non-host adapted (Cooper, 1994), thus the actual source of infection was not clear, if came from farm workers, feeders or water. Due to short time and very weak budget of this study, it was not possible to carry an epidemiological investigation to determine the source of this serovar.

The utility of the Salmonella Chromogenic Medium as specific and time-saving method for the detection and presumptive identification of *Salmonella* species was demonstrated. It was used after enrichment in selenite-f-broth, but it can directly be used if number of cells in the clinical sample is expected to be high. Because the medium is designed to isolate *Salmonella* with high specificity and sensitivity, the result obtained is considered true. The *Salmonella* detection potential of this medium is based on the presence of the Chromogenic substrate, Magenta-caprylate which is hydrolyzed by *Salmonella* species to produce magenta colonies. This is beside X-gal which was incorporated to visualize *β*-glactosidase-producing organisms as blue colonies. The Chromogenic mixture plus sodium citrate inhibit Gram-positive bacteria, *Proteus* and *Coliforms* (Miles *et al.,* 1992). This type of medium is highly recommended to be used by non-expert hands as it depends on observation of specific color of colonies. It can be said that one day is enough to start treatment, either after direct culture on this medium or after enrichment and sub-culture on the solid Chromogenic medium. This medium is more selective and the colonies are specially colored compered to conventional media. The owner of the positive farm was extremely happy after informing him, two days post sampling, that he

should start mass treatment using Ciprofloxacin, which was very successful approach. Odonoughe (1993) found more specific and sensitive detection of *Salmonella* from foods for Salmonella Chromogenic media compered to conventional ones.

Determination of *Salmonella* to the species level took one day using few sugar fermentation tests. The biochemical results showed that the *Salmonella* isolates belong to one strain.

The antibiotic sensitivity test that was carried out for the *Salmonella* isolates in this study displayed one antibiotic susceptibility pattern, as all isolates were sensitive to all antibiotics tested except Tetracycline and Co-trimoxazole. This may indicate that all of the isolates belong to one strain of *S*. Enteritidis. This finding of drug susceptibility is considered in accordance with previous reports (Kheir Eldin *et al*., 1987; Roliniski *et al*., 1994; Esaki *et al*., 2004). During a 9-year study, a small proportion of resistant strains were found within *S*. Enteritidis, 2.3% showing resistance to at least one antimicrobial drug and 0.9% to three or more (Goodnough and Johnson, 1991). Prevalence of resistant strains in southern Italy was similar to that in other European countries, such as England and Wales (Threlfall *et al*., 1997) and the Czech Republic (Sramova *et al.,* 1999); however, it was lower than prevalence detected from 1987 to 1993 in Greece, where up to 67.4% of strains of *S*. Enteritidis from human and non-human sources were resistant to antibiotics and the resistance rate increased steadily until 1991 (Tassios *et al.,* 1997). Tetracycline had been used as a growth enhancer in food producing animals until 1998, when its use was banned (Saikia *et al*., 1986). It has also been used as a therapeutic agent, thus the high level of resistance to Tetracycline is not surprising (Irwin *et al*., 1989).

Conclusions:

An outbreak of *Salmonella* infection in one layer chicken farm in Shambat area was detected, while farms of other areas of Khartoum State were negative. The serovar caused the outbreak was identified as *Salmonella* Enteritidis, which can infect man through consumption of contaminated eggs.

All *Salmonella* isolates were found sensitive to Amikacin, Ceftizoxime, Ciprofloxacin, Gentamicin, Ampicillin\Sulbactam, Piperacillin\Tazobactam, Cefotaxime and Chloramphenicol and resistant to Tetracycline and Co-trimoxazole.

The Salmonella Chromogenic Medium used in this study was found useful as rapid and simple detection tool of *Salmonella* in clinical samples.

Recommendations:

Because of specificity, simplicity and rapid results obtained from Salmonella Chromogenic Medium, it is recommended to be used in diagnostic Labs in hospitals as well as in animal farms, so that early diagnosis of *Salmonella* infections can be achieved, which will very much help in the proper treatment and control of the disease.

A large size of epidemiological studies to isolate *Salmonella* from animals and their environment and to perform strain typing using molecular techniques is recommended.

References

Aamlid, K.H.; Lee, G.; Price, R. G.; Richardson, A.C.; Smith, B.V. and Taylor, S.A. (1989). Development of improved chromogenic substrates for the detection and assay of hydrolytic enzymes. *Chem. Ind*. **21:** 106–108.

Al Obaidi, A.S.; AlAni, I.A.; AlSoudi, K.A.; and Abdul-Gani, Z.G *Agric. Water. Res. Anim. Prod.* **6** (2)**:** 51-61.. (1992). Incidence of *Salmonella* in a flock of chicken native to Iraq. *J.*

Anonymous, G.A. (1990). USDA task force reports on 10 *S. enteritidis* outbreaks. *Food Chem. Ns*. **32:** 43-44.

Bailey, J.S. (1988). Integrated colonization control of *Salmonella* in poultry. *Poult. Sci*. **67:** 928-932.

Barons, E.C. (1996). *Medical Microbiology,* 4th ed. University of Texas Medical Branch.

Barrow, G.I. and Feltham, R.K.A. (1993). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 3rd ed. Cambridge University Press, Cambridge, U.K.

Barrow, P.R.; Barrow, G.C.; Mead, C.; Wray and Duchet-Suchaux, M. (2003). Control of food poisoning in poultry. *Worlds Poult. Sci. J.* **59:** 373-383.

Bauer, A.W.; Kirby, W.M.; Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by standardized single disc method. *Am. J. Clin. Pathol.* **45:** 493-496.

Beaumonal, C.; Beaumont, J.; Protais, J. F.; Guillot, P.; Colin, K.; Proux, N. and Pardon, P. (1999). Genetic resistance to mortality of one day-old chicks and carrier-state of hens after inoculation with *Salmonella enteritidis*. *Av. Pathol.* **28:** 131-135.

Birch Hirschfeld, L. (1935). The Bacteriology of the Enterobacteriaceae. *Acta. Pathol. Scand*. **25:** 117-626.

Bolder, N.M.; Wagenaat, F. F.; Pultrulan, K.T. and Sommer, M. (1990). The effect of flavophospholipol (Flavomycin7) and salinomycin sodium (Sacox7) on the excretion of *Clostridium perfringens*, *Salmonella enteritidis* and *Campylobacter jejuni* in broilers after experimental infection. *Poult. Sci.* **78:** 1681-1689.

Bouvet, P.J.; Fougerat, I.; Guesnier, F.; Guiber, V.; Lenormand, P. and Grimont, P.A.D. (2002). Human Salmonellosis Surveillance in France: recent data from the National Reference Center. In: Colin, P., Clement, G. (Eds.), *International Symposium on Salmonella and Salmonellosis*, pp. 411-416.

Bryan, F.L. Donle, M.P. (1995). Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry .*J. Food Protect,* **58:** 328-344.

Buxton, A. and Fraser, G. (1977). Immunology, bacteriology, mycology and diseases of fish and laboratory methods. *Animal Microbiology*, 1st ed. Blackwell, publishing, pp. 103-105.

Carter, G.R. and Wise, J. (2004). *Essentials of Veterinary Bacteriology and Microbiology*, 6th ed. Blackwell, publishing.

CDC (2000). Outbreaks of *Salmonella enteritidis* infection associated with eating raw or undercooked shell eggs in United States.

Cheesbrough, M. (2000). *District Laboratory Practice in Tropical Countries*. Part 2. Cambridge University Press, Cambridge.

Cooper, G.L. (1994). *Veterinary Microbiology and Microbial Disease*. Blackwell, publishing, pp. 114-116.

Cowan, S.T. and Steel, K.J. (1985). *Cowan and Steel's Manual for Identification of Medical Bacteria*, 2nd ed. Cambridge University Press, London, U.K.

Cruickshank, A. (1972). *Medical Microbiology, A Guide to Lab. Diagnosis and Control of Infection*. Nary Bulletin London, pp. 123-143.

Eberth, C.J. (1980). *Principles of Bacteriology and Immunology*, 4th ed. Blackwell, publishing, pp 82-85.

Esaki, H.; Morioka, K.; Ishihara, A.; Kojima, S.; Shiroki, Y. and Takahashi, T. (2004). Antimicrobial susceptibility of *Salmonella* isolated from cattle, swine and poultry (2001-2002): report from the Japanese Veterinary Antimicrobial Resistance Monitoring Program. *J. Microb.***16:** 105-116.

Ezdihar, A.A. (1996). *Isolation and Characterization of Salmonella from Domestic Fowl and its Environment in Kordofan State.* MSc. Thesis, University of Khartoum, Sudan.

Felix, A. and Pitt, R.M. (1943). A new antigen of *B. typhosus* it's relation to virulence and active and passive immunization. *Lancet*. **11:** 186-191.

Fertado, G.K.; Wall, H.S. and Casemore, D.P. (1998). Outbreaks of waterborne infectious intestinal disease in England and Wales (1992-1995). *Epidemiol. Infec*. **21:** 109-119.

Gast, R.K. (1997). Detecting infection of chickens with recent *Salmonella pullorum* isolates using standard serological methods. *Poult. Sci*. **76:** 17-23.

Goodnough, M.C. and Johnson, E. A. (1991). Control of *Salmonella enteritidis* infections in poultry by polymyxin B and trimethoprim. *Appl. Envi. Microbiol.* **57:** 785-788.

Hafez, H.M. (2005). Government regulation and control of some important poultry diseases. *World's Poult. Sci. J*. **61:** 574-575.

Hafez, H.M. and Jodas, S. (2000). *Salmonella in Domestic Animals*. International Wallingford, U.K. pp. 133-155.

Hibba, K. (2007). *Isolation and Identification of Salmonella species from Chickens in Khartoum State* .MSc. Thesis, University of Khartoum, Sudan.

Hisham, E.M. (2010). *Isolation and Identification of Salmonella from the Environment of Traditional Poultry Farms in Khartoum North*. MSc. Thesis, University of Khartoum, Sudan.

Holt, N.R.; Krieg, P.H.A.; Sneath, C. and Williams, S.T. (1994). *Bergey's Manual of Determinative Bacteriology*, 9th ed. Williams and Wilkins Company, Baltimore, pp. 787-791.

Irwin, R.J.; Mcewen, A.; Clarke, R.C. and Meck, A.H. (1989). The prevalence of vero-cytotoxin-producing *Escherichia coli* and

antimicrobial resistance patterns of non-vero cytotoxin producing *Escherichia coli* and *Salmonella* in Ontario broiler Chickens. *Can. J. Vet. Res*. **53:** 411-418.

Janda, J.M. and Abbott, S.L. (2006). *The Enterobacteria,* ASM Press.

Jardy, N. and Michard, J. (1992). *Salmonella* contamination in raw feed components. *Microbiol. Alimen. Nutr*. **10:** 233-240.

Kauffman, F. (1966). Two biochemical subdivisions of the genus *Salmonella*. *Acta Patholo. Scand*. **49:** 393-396.

Khan, A.Q. (1969). *Animal Salmonellosis in the Sudan*. A PhD. Thesis, University of Khartoum, Sudan.

Kheir El-Din, A.M.W.; Hamed, O.M. and Mashhoor, M.M. (1987). Chemotherapeutic sensitivity testing and pathogenicity of *Salmonella* isolated from poultry farms in Egypt. *Vet. Med. J.* **35:** 315-323.

Koneman, W.G.; Allen, D.S.; Janda, M.W.; Sclreckenberger, C.P and Win, C.W. (1997). *Color Atlas and Text Book of Diagnostic Microbiology,* 5th ed. Williams and Wilkins, Baltimore, pp. 125-127.

Koo, F.C.; Peterson, C.W.; Houston, R. and Molina, N.C. (1984). Pathogenesis of experimental salmonellosis: Inhibition of protein synthesis by cytotoxin. *Epidemiol. Infect.***43:** 93-100.

Le Minor, L. and Popoff, M.Y. (1987). Designation of *Salmonella enterica*. *Int. J. Sys. Bacteriol.* **37:** 465-468.

Madden, J. M. (1990). *Salmonella enteritidis* contamination of whole chicken eggs. *Dairy Food Environ. Sanitation.* **10:** 268-270.

Manafi, M. (2000). New developments in chromogenic and fluorogenic culture media. *Int. J. Food Microbiol*. **60:** 205-218.

Merchant, I.A. and Packer, K.A. (1967). *Veterinary Bacteriology and Virology,* 7th ed. Williams and Wilkins, Baltimore, pp. 78-89.

Miles, R.J.; Siu, E. L.T.; Carrington ,C.; Richardson, A.C.; Smith, B.V. and Price, R.G. (1992). The detection of lipase activity in bacteria using novel enzyme substrates. *FEMS. Microbiol. Lett*. **90**: 283–288.

Minor, L.L.E. (1984). Genus III *Salmonella lignieres* In: Kreig,P.H.A. and Holt, N.R. *Bergery's Manual of Systematic Bacteriology*, Williams and Wilkins, Baltimor, London, pp. 427-458.

Munnich, D. (1973). The treatment of salmonellosis with trimethoprim/ sulphamethoxazole and their combination with polymyxin B in adult patients. *Chemotherapy*. **18:** 119-129.

Murray, C.J. (2005). *Salmonella* in the environment. *Rev. Sci. Tech.* **22:** 247-249.

Nurmi, E. and Rantala, M. (1973). *New Aspects of Salmonella.* Williams and Wilkins, Baltimore, pp. 243-250.

Odonoughe, D.E. (1993). Comparison of the MSRV method with an in-house conventional method for the detection of *Salmonella* in various high and low moisture foods. *Lett. Appl. Microbiol*. **17:** 174–177.

Orhan, G. and Guler, L. (1993). Bacteriological and serological identification of *Salmonella* species in the organs and feces of chicken eggs and feed. *Poult. Sci.* **4:**15-20.

Pan, G.Y.; Liu, L.; Chen, J.D. and Luo, D.Y. (1993). Isolation and identification of enterobacterial strains from chicks. *Avian Dis*. **28:** 272-275.

Poppe, C. (1994). *Salmonella enteritidis* in Canada. *Intern. J. Food Microbiol.* **22:**1-5.

Poppe, C. and Gyles, C.L. (1987). Relation of plasmids to virulence and other properties of *Salmonella* from avian sources. *Avian Dis*. **31:** 844-854.

Poupart, M.C.; Mounier, M.; Denis, F.; Sirot, J.; Couturier, C. and Villeval, F. (1991). Abstracts of the Fifth European Congress of Clinical Microbiology and Infectious Diseases. A new chromogenic ready-to-use medium for *Salmonella* detection, *Abstr*. 1254.

Quinn, B.K.; Markey, M.E.; Carter, W.J.; Donnelly and Leonard, F.C. (2002). *Veterinary Microbiology and Microbial Disease*. Blackwell publishing, pp. 17-20.

Quinn, P.J.; Markey, B.K.; Carter, M.E.; Donnelly, W.J. and Leonard, F.C. (2008). *Veterinary Microbiology and Microbial Disease*. Blackwell publishing, pp. 60-65.

Rabsch, W.; Rabsch, B.M.; Hargis, R.M.; Tsolis, R.A.; Kingsley, K.H.; Hinz, H.; Tschape and Baumler, A. (2000). Competive exclusion of *Salmonella enteritidis* by *Salmonella gallimarum* in poultry. *Emerging Infec. Dis*. **7:** 443-448.

Reynolds, D.J.; Davies, M. and Wray, C. (1997). Evaluation of combined antibiotics and competitive exclusion treatment in broiler breeder

flocks infected with *Salmonella enterica* serovar *enteritidis*. *Avian. Pathol.* **26:** 83-93.

Roliniski, Z.; Wernicka-Furmaga, R. and Kowalski, C. (1994*)*. Activity of flumaquine against *Salmonella* species *in-Vitro* and the treatment of experimental infection in mice. *Vet. Med*. **44:** 416-465.

Ruiz, J.; Lunez, M.; Diaz, J.; Lorente, I.; Perez, J. and Gomez, J. (1996). Comparison of five plating media for isolation of *Salmonella* species from human stools. *J. Clin. Microbiol*. **34**: 686–688.

Ryan, K.J. and Ray, C.G. (2004). *Medical Microbiology, 4th ed*. McGraw Hill, pp. 362–368.

Saikia, G.K. and Patgiri, G.P. (1986). *Salmonella* serotypes isolated from poultry in Assam. India. *J. Microbiol. Infect. Dis*. **7:** 83-85.

Seuna, E. and. Nurmi, E. (1980). Therapeutical trials with antimicrobial agents and cultured fecal microflora in *Salmonella infantis* infections in chickens. *Poult. Sci*. **58:** 1171-1174.

Sharp, J.C.M. (1988). Salmonellosis and eggs: more information needed, meanwhile avoid raw egg. *Br. Med. J.* **297:** 1557-1558.

Shivaprasad, H.L.; Nagarja, B.S. and Williams, J.E. (1998). Pathogenesis of *Salmonella eneritidis* infection in laying chickens. *Avian Dis.* **34:** 548-557.

Soumet, G.; Ermel, N.; Rose, P.; Drouin, G.; Salvat and Colin, P. (1999). Evaluation of multiplex assay for simultaneous identification of *Salmonella spp*., *Salmonella enteritidis* and *Salmonella typhimurium*

from environmental swabs of poultry houses. *Appl. Microbiol.* **28:** 113-117.

Sramova, H.; Karpiskova, R.; Dedicova, D.; Sisak, F. and Rychlik, I. (1999). Properties of *Salmonella* isolates in the Czech Republic. *Epidemiol. Microbiol. Immunol*. **48:** 111-116.

Tassios, P.T.; Markogiannakis, A.; Vatopoulos, A.C.; Katsanikou, E.; Velonakis, E.N. and Kourea-Kremastinou, J. (1997). Molecular epidemiology of antibiotic resistance of *Salmonella enteritidis* during a 7-year period in Greece. *J. Clin. Microbiol*. **35:** 13-21.

Threlfall, E.J.; Ward, L.R.; Skinner, J.A. and Rowe, B. (1997). Increase in multiple antibiotic resistance in non typhoidal *salmonellas* from human in England and Wales: a comparison of data for 1994 and 1996. *Microb. Drug Resist.* **3:** 263-266.

Waltman, W.D.; Horne, A.M. and Pirkle, C. (1993). Influence of enrichment incubation time in the isolation of *Salmonella*. *Avian Dis.* **37:** 884-887.

Williams, H. and Tucker, J.F. (1975). The effect of antibiotic therapy on the fecal excretion of *Salmonella typhimurium* by experimentally infected chickens. *J. Hyg*. **75:** 275-292.

Williams, J.F. and Benson, S.T. (1978). Survival of *Salmonella typhimurium* in poultry feed and litter at three temperatures. *Avian Dis.* **22:** 742-747.

Wilson, M.A. and Nordholm, G.E. (1995). DNA fingerprint analysis of standard, intermediate and variant antigenic types of *Salmonella*

enterica subspecies *enterica s*erovar *gallinarum*. *Avian Dis*. **39:** 594-598.

Wray, C. and Wray, A. (2000). *Salmonella* in domestic animals. *CAB. Int.* **32:** 407-427.

Yagoub, I.A. and Mohammed, T.E. (1987). Isolation and identification of *Salmonella* from chickens in Khartoum province of the Sudan. *Brit. Vet. J.***143:** 537-540.

Zhang-Barber, A.K. and Turner, P.A. (1999). Vaccination for control of *Salmonella* in poultry. *Poult. Sci*. **17:** 2538-2545.

Zhao, B.; Villena, R.; Sudler, E.; Yeh, S.; Zhao, D.G.; White, D. W.; anger, M. and Meng, J. (2001). Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the greater Washington. *Appl. Envi. Microbiol.* **67:** 5431-5436.